Optimum conditions for asparaginase extraction from *Pisum sativum* subspp. Jof

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Abstract

Asparaginase was extracted from plant parts of *Pisum sativum* subspp. Jof collected from a field crop. Asparaginase activity was detected in seeds, stems and leaves extracts. Enzyme activity was higher in seeds extracts (30.0 U/ml) compared with leaves extracts (26.4 U/ml) and stems extracts (16.1 U/ml), respectively. Optimum conditions for the activity of crude asparaginase extracted from plants seeds were studied. Results showed maximum activity of asparaginase was achieved when the enzyme was incubated with 200 mM of asparagines in a ratio of 1:3 (V/V) at 37 °C for 30 minutes in the presence of 0.05 M of potassium phosphate buffer solution at pH 8. Asparaginase activity was equal to 602.6 U/ml under optimum conditions.

Keywords: asparaginase; *Pisum sativum*; enzyme conditions

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Introduction

L-asparaginase (E.C.3.5.1.1) is the enzyme that catalyses the hydrolysis of the amide group of L-asparagine releasing L-aspartate and ammonia. The enzyme plays important roles both in the metabolism of all living organisms as well as in pharmacology (Borek and Jaskolski, 2001). The action of asparaginase plays a major role in the cellular nitrogen metabolism of both prokaryotes and eukaryotes (Yossef and Al-Omar, 2008). The two largest and well characterized families of asparaginase include bacterial-type and plant-type asparaginases (Michalska and Jaskolski, 2006). In *Pisum sativum* and many other legumes asparaginases liberate from asparagine the ammonia that is necessary for protein synthesis. There are two groups of such proteins, namely, potassium-dependent and potassium-independent asparaginases. Both enzymes have significant levels of sequence similarity (Lough et al., 1992.

L-asparaginase is a therapeutically important protein used in combination with other drugs in the treatment of acute lymphocyte Leukemia (mainly in children, Hodgkin disease, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticlesarcoma and melanosarcoma (Stecher, 1999; Verma, 2007). In Food industry, it was used to determine and eliminate acrylamide from bread using gene technology by degrading asparagines, the precursor of acrylamide. Also, in biosensors it is used for monitoring asparagine levels in mammalian and hybridoma cells (Taeymans, et al., 2005). The main objective of this study is to determine optimum conditions for the extraction of asparaginase from *P. sativum subspp. Jof.*

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Materials and Methods

Sample collection

Plant parts (leaves, stems and seeds) of *Pisum sativum* were collected during season 2011 from field of plant crops.

Extraction of asparaginase from different plants parts

Extraction of asparaginase from plant parts was achieved according to Chang and Farnden (1981) by homogenizing 10 g of plant parts with three volumes of 0.05 M potassium phosphate buffer, pH 8.0 containing 1.5 M sodium chloride, 1 mM PMSF, 1 mM EDTA, and 10% (w/v) glycerol, then centrifuged at 10000 rpm for 20 minutes. Supernatant was regarded as crude enzyme.

Enzyme assay

Asparaginase was assayed according to Nesslerization method based on the conversion of L-asparagine to Ammonia and L-asparatate, as described by Ren *et al*. (2010).

Protein concentration in plant extracts and enzyme concentrates was determined according to Bradford method (1976).

Determination of optimum conditions for crude asparaginase activity

Effects of different factors on the activity of crude asparaginase extracts were studied by determining the optimum conditions for enzyme activity according to Bello et al. (2011). These factors include substrate concentration, time of reaction, pH of extraction buffer, reaction temperature, type of extraction buffer, and enzyme: substrate ratio.

Effect of substrate concentration

Effect of substrate concentration on the activity of crude asparaginase was determined by incubation of crude enzyme extract with different L-asparagine concentrations (10, 50, 100, 150, 200, and 250 mM). Then asparaginase activity was determined.

Effect of reaction time

Effect of reaction time for asparaginase was determined by incubation of the reaction mixture for a different period of time (15, 30, 45, 60, and 90 minutes) at 37 °C. Then enzyme activity was determined.

Effect of buffer pH

In order to determine the optimal pH for crude asparaginase activity, pH of the reaction mixture was adjusted to different values range (7.5, 8.0 and 8.5). Then enzyme activity was determined.

Effect of Temperature

Optimal temperature for crude asparaginase activity was determined by incubation of the reaction mixture at different temperatures (25, 30, 37 and 40 °C). Then enzyme activity was determined.

Effect of extraction buffer

Effect of the type of extraction buffer on the enzymatic activity was studied by using 0.05 M Tris-HCl buffer solution at pH 8.0, and 0.05 M potassium phosphate buffer at pH 8.0. Then enzyme activity was determined.

Effect of Enzyme: substrate ratio

Effect of enzyme: substrate ratio on asparaginase activity was determined by using different ratios (1:1, 1:2 and 1:3) of enzyme: substrate. Then enzyme activity was determined.

Results

Asparaginase activity was detected in extracts of seeds, leaves, and stem tissues of *Pisum sativum*. Results indicated in Table (1) showed that maximum asparaginase activity (30 U/ml) and total activity (420 U) was noticed in plant seeds extracts, which was much greater

Table 1
Asparaginase activity and specific activity in the extracts of plant parts of *Pisum sativum*

Plant part	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specific Activity (U/mg)	Total activity (U)
Seeds	14	30.0	6.4	4.6	420
Leaves	14	26.4	4.8	5.5	369.6
Stems=	14	16.1	4.8	3.0	225.4

than those in other plant parts extracts (leaves and stems).

Optimum substrate concentration

Results mentioned in Fig. (I) showed that the activity of asparaginase in seeds extracts was increased gradually with the increase in L-asparagine concentration. Maximum activity of asparaginase was obtained when the substrate concentration was 200 mM. At this concentration, asparaginase activity was 332.7 U/ml, and at this substrate concentration asparaginase activity reached the steady state.

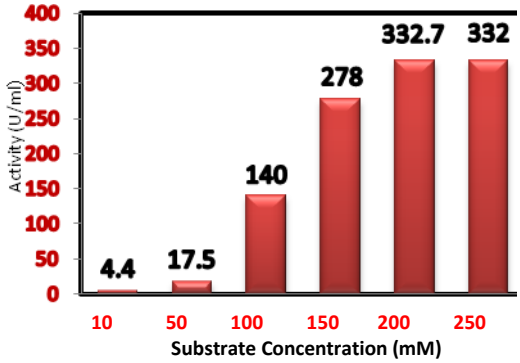

Fig. I. Effects of substrate (asparagine) concentration on the activity of asparaginase extracted from seeds of *Pisum sativum* incubated at 37 °C for 30 minutes, pH=8

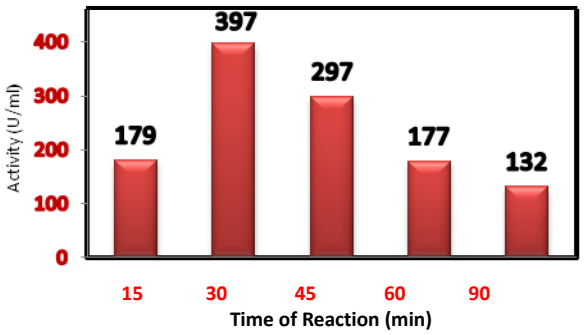

Fig. II. Effect of reaction time on asparaginase activity extracted from seeds of *Pisum sativum* using 200 mM asparagine, potassium phosphate buffer pH 8 incubated at 37 °C

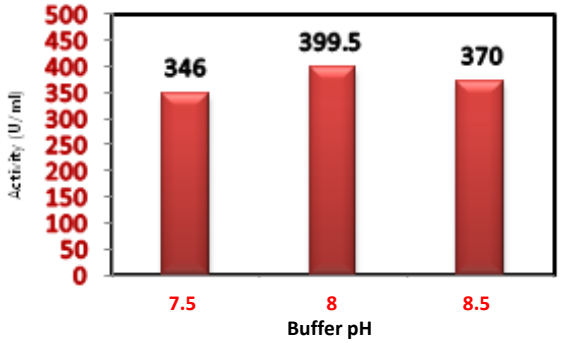

Fig. III. Effect of buffer pH on the activity of asparaginase extracted from seeds of *Pisum sativum,* 200 mM aspargine, incubated at 37 °C for 30 min.

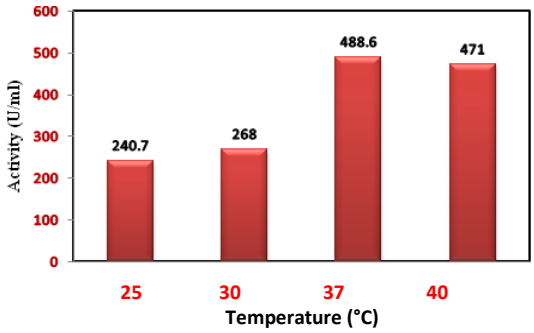

Fig. IV. Effect of temperature on the activity of asparaginase extracted from seeds of *Pisum sativum*

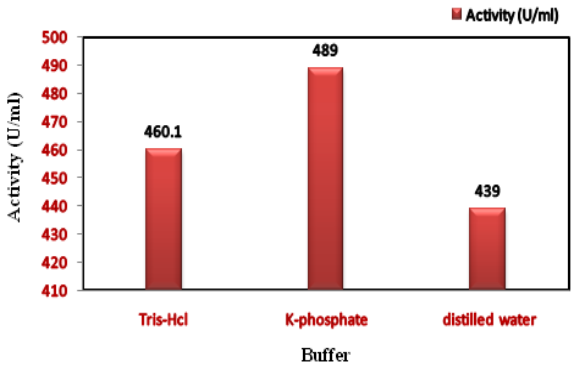

Fig. V. Effect of buffer solution type at pH 8 on the activity of asparaginase extracted from seeds of *Pisum sativum* incubated at 37 °C for 30 min.

Optimum reaction time

As Fig. (II) shows, the optimum reaction time for asparaginase activity was 30 minutes. After this period, the enzyme activity reached 397 U/ml, and then the activity decreased with an increase in reaction time.

Optimum buffer pH

Result illustrated in Fig. (III) showed that maximum asparaginase activity was obtained when pH of the reaction mixture was adjusted to 8.0. At this value, the enzyme activity was 399.5 U/ml.

Optimum temperature

Results illustrated in Fig. (IV) showed that the maximum activity of asparaginase was obtained when the temperature of the reaction mixture was 37 °C. At this temperature, asparaginase activity increased to 488.6 U/ml. The increase or decreases in the incubation temperature above or below the optimum temperature caused a decrease in enzymatic activity.

Optimum extraction buffer

Results mentioned in Fig. (V) showed that the maximum activity of asparaginase was obtained when potassium phosphate buffer solution was used. By using this type of extraction buffer, asparaginase activity in seeds extracts reached 489 U/ml.

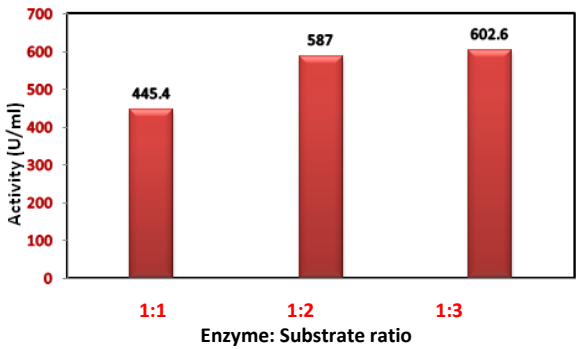

Fig. VI. Effect of enzyme: substrate ratio on the activity of asparaginase extracted from seeds of *Pisum sativum*

Optimum Enzyme: substrate ratio

Effect of enzyme: substrate ratio on the activity of asparaginase extracted from *Pisum sativum* seeds was studied by using different ratios of enzyme: substrate. Results mentioned in Fig. (VI), showed that asparaginase activity reached 602.6 U/ml when the ratio of enzyme: substrate was 1:3.

Discussion

Among different plants, *Pisum sativum* is a suitable source for asparaginase which its activity was detected in different plant parts (leaves, stems and seeds). This is usually due to the high levels of asparaginase expression in developing seeds where asparaginase supplies 50-70% of the required nitrogen as mentioned by Michalska et al. (2006). Accordingly, plant seeds extracts were used to study the optimum conditions for enzyme activity and determine if the enzyme specificity was towards its substrate (asparagine). Increasing the substrate concentration increases the rate of reaction (enzyme activity). However, enzyme saturation limits reaction rates. Experimentally, the amount of the enzyme is kept constant and the substrate concentration is then gradually increased. The reaction velocity will increase until it reaches a maximum. Rate of the reaction usually is proportional to the incubation time with asparagine until it reaches a steady state. It is well known that time-scale is an important factor in determining the enzyme activity as mentioned by Dalaly, 1990). pH is a factor independent for asparaginase activity because the ionizable groups of enzymes are affected by the pH value. Changes in pH may not only affect the shape of an enzyme but it may also change the shape or charge properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis. It has been reported that most plant asparaginases have their maximum activity in alkaline pH, and with pH range of 7.5, 8.0, and 8.5 as mentioned by Oza, 2009). Reaction temperature is one of critical factors affecting asparaginase activity. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to 20% in

the results. Above a certain temperature, enzyme activity decreases because of enzyme denaturation. In general, it was found that asparaginase from most organisms have maximum activity at 37 °C (Abdel Hameed, 2005). On the other hand, the composition of buffer solution has significant effects on enzymatic activities. Potassium phosphate buffer solution was adaptive or inducer for plant asparaginase activity, acting as a source of potassium ion for activation of asparaginase. Hence, asparaginase obtained from seeds of *P. sativum*, was potassium dependent as mentioned by Sodek et al. (1980) and Michalska and Jaskoliski, (2006).

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